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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 99,576-A)

In the Application of:	)	
	)	
Mohammad S. Nasir and	)	Examiner: Deborah A. Davis
Michael E. Jolley	)	
	)	
Serial No.: 09/903,061	)	Art Unit: 1641
	)	
Filed: July 11, 2001	)	
	)	
For: Fluorescence Polarization-Based	)	
Homogeneous Assay for Deoxynivalenol	)	
In Grains	)	

DECLARATION OF MICHAEL E. JOLLEY
PURSUANT TO 37 C.F.R. § 1.132

I, Michael E. Jolley, residing at 34469 North Circle Drive, Round Lake, Illinois 60073, hereby declare:

1. I am founder, part owner, and scientific director of Diachemix LLC, the owner of this patent application, Serial No. 09/903,061.

2. I received a PhD in carbohydrate chemistry from the University of Birmingham (United Kingdom) in 1972.

3. I have been working with fluorescence polarization techniques for over 25 years.

4. I am a listed inventor on many issued U.S. patents relating to fluorescence polarization and have authored or co-authored many articles on fluorescence polarization.

5. Fluorescence polarization assays are based on the fundamental principal that, due to the molecular rotation occurring during the fluorescence lifetime of the excited state, smaller molecules tend to have smaller fluorescence polarization values (because they rotate faster), and larger molecules tend to have larger fluorescence polarization values (due to their slower rotation).

As a result, changes in molecular size, e.g., due to reaction, can often be monitored as changes in fluorescence polarization.

6. In fluorescence polarization assays, the binding of a fluorescently labeled antigen with an antibody increases the effective molecular size and, thus, can often be detected as an increase in fluorescence polarization.

7. However, it is not the case that the binding of any fluorescently labeled antigen with an antibody will necessarily produce a detectable change in fluorescence polarization. In practice, sometimes the binding can be detected as a change in fluorescence polarization; sometimes it cannot.

8. For example, the "propeller effect" can prevent any change in fluorescence polarization from being observed. The propeller effect is caused by the uncoupling of the fluorophore from the antigen/antibody binding site, due to a long, flexible linkage between them.

9. Labelling the antigen to form the fluorescent tracer can also interfere with its ability to bind with the corresponding antibody.

10. Sergei A. Eremin and David S. Smith, "Fluorescence Polarization Immunoassays for Pesticides," *Combinatorial Chemistry & High Throughput Screening*, vol. 6, pp. 257-266 (2003), attached hereto as Exhibit A, is an article regarding fluorescence polarization assays for pesticides. Professor Sergei Eremin is one of the world's foremost experts in the field. That article points points out some of the difficulties of developing fluorescence polarization assays and concludes that "the development of suitable antibodies and the design of fluorescein-labeled tracers for the FPIA of pesticides remain objectives for research." Although this statement refers to pesticides, in my opinion, the statement is also applicable to other analytes.

11. C.M. Maragos, M.E. Jolley, and M.S. Nasir, "Fluorescence polarization as a tool for the determination of deoxynivalenol in wheat," *Food Additives and Contaminants*, vol. 19, pp. 400-407 (2002), attached as Exhibit B, reports work in which I was involved to develop a fluorescence polarization assay to detect deoxynivalenol (DON) in grains. As reported in that article, three monoclonal antibodies that had been developed for ELISA were tried in a fluorescence polarization assay for DON, but only two of them worked. Unexpectedly, the antibody that was most sensitive in the ELISA format did not work in the fluorescence polarization assay, which used fluoresceinamine isomer II as the fluorophore. Apparently, this antibody either did not bind to the tracer or bound to the tracer without producing a detectable change in fluorescence polarization.

12. Chris M. Maragos and Ronald D. Plattner, "Rapid Fluorescence Polarization Immunoassay for the Mycotoxin Deoxynivalenol in Wheat," *J. Agric. Food Chem.*, vol. 50, pp. 1827-1832 (2002), attached as Exhibit C, reports that the antibody that was the most sensitive in the ELISA format could be used in a fluorescence polarization assay for DON by using 4'-(aminomethyl) fluorescein instead of fluoresceinamine isomer II.

13. I hereby declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 11/14/03

Signed: _____
Michael E. Jolley